σ is stress, ε is strain, and θ is the phase shift. We found that both the viscous modulus and the elastic modulus were higher in the AI KO at high frequencies. These results suggest that WT passive tension levels rely on an intact A-I junction; the removal of this region results in increased titin stiffness.

1747-Pos Board B477

Myocardial Titin: An Important Modifier of Cardiac Stiffness

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Background: A well-established function of titin is the determination of passive tension ($F_{passive}$) in myocardium. Modifications to the elastic titin region have been suggested to contribute to left ventricular (LV) diastolic dysfunction in heart failure (HF). Titin-based stiffness can be modulated by isoform switch or phosphorylation.

Results: We find that titin-isoform switch accounts for a significant amount of myocardial stiffness modulation, giving rise to increased or reduced $F_{passive}$ in different types of heart failure. In addition, both acute and chronic modulations of cardiomyocyte $F_{passive}$ occur via altered titin phosphorylation. Cyclic AMP-dependent protein kinase-A, cGMP-dependent protein kinase-G, and extracellular signal-regulated kinase-2 phosphorylate titin at a cardiac-specific domain, the N2Bus; this phosphorylation results in a reduction in cardiomyocyte $F_{passive}$ in various species. PKCα phosphorylates the PEVK-domain of titin, which increases $F_{passive}$ of normal mouse cardiomyocytes, but does not significantly alter $F_{passive}$ of cardiomyocytes obtained from a dog HF model. Calcium/calmodulin-dependent protein kinase-II (CaMKII) is the first kinase found to phosphorylate both the N2Bus and the PEVK-domain. This phosphorylation reduces cardiomyocyte $F_{passive}$, as demonstrated in skinned mouse cardiomyocytes incubated with recombinant CaMKIIδ. Moreover, $F_{passive}$ is elevated in cardiomyocytes of CaMKIIγ/δ double knockout mice and reduced in those of CaMKIIδ-overexpressing transgenic mice. In both human and experimental HF, a global titin phosphorylation deficit is observed, but site-specific titin phosphorylation can be increased or decreased in HF, presumably depending on the activity and expression level of the relevant kinases.

Conclusion: Titin phosphorylation may have beneficial effects in the heart via reducing myocardial diastolic stiffness and improving ventricular filling. Altered titin phosphorylation in HF may severely affect $F_{passive}$ and compromise cardiac function. The degree, to which the different protein kinases contribute to alterations in diastolic passive stiffness, needs to be determined.

1748-Pos Board B478

A Gain-Of-Function Mutation in Cardiac Myosin Binding Protein-C Increases Viscoelastic Load and Slows Shortening Velocity in Myocytes from Transgenic Mice

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Cardiac myosin binding protein C (cMyBP-C) is a sarcomeric protein involved in the regulation of cardiac muscle contraction. Effects of cMyBP-C on contraction are thought to be mediated in part by limiting the interactions of actin and myosin to slow myocyte shortening velocity and power output. Although interactions with myosin S2 on the thick filament have been proposed as a way in which cMyBP-C could limit shortening velocity (e.g., by creating a drag force on myosin heads), interactions of cMyBP-C with actin could also account for slowed shortening velocity. For instance, cMyBP-C could create a drag that opposes filament sliding by transiently linking thick and thin filaments together. To explore this possibility we created transgenic mice that express a mutant cMyBP-C with a point mutation (L348P) located in a conserved sequence within the regulatory M-domain that increases cMyBP-C binding to actin in vitro (Bezold et al, JBC, 2013). We reasoned that if the mutation also enhanced binding to actin in sarcomeres then shortening velocity would be slowed in myocytes from L348P mice. Results show that transgenic mice expressing the L348P mutation are viable and that L348P cMyBP-C is expressed in sarcomeres. Permeabilized myocytes from transgenic mice showed altered force production including reduced maximal force and enhanced Ca^{2+} sensitivity of tension. Shortening velocity and power output were significantly reduced whereas passive stiffness and myocyte visco-elasticity were significantly increased. Together these data are consistent with the idea that cMyBP-C creates an internal load in the sarcomere by binding to actin. This

work supported by NIH R01 HL080367 (SPH) and an AHA graduate fellowship (KLB).

1749-Pos Board B479

Exercise-Induced Enhancement of Cardiac and Sarcomere Performance is Larger in Male than in Female MYBPC3 Mutation Heterozyous Knock-In Mice

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Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disorder. Mutations in the gene (*MYBPC3*) encoding cardiac myosin binding protein C (cMyBP-C) are a frequent cause of HCM. Clinical as well as animal-model studies have reported sex-related differences in HCM disease onset and severity. In addition, it has been established that physiological stimuli such as exercise may elicit a sexually dimorphic cardiac response. However, less attention has been paid to the sex-specific differences in the cellular pathophysiologic mechanisms underlying HCM. Therefore, we studied functional properties of the heart and sarcomeres in male and female sedentary and exercise (exposed to 8 weeks voluntary wheel running) mice.

Echocardiography and isometric force measurements in mechanically isolated left ventricular (LV) membrane-permeabilized cardiomyocytes were performed in Wild-type (WT) and heterozygous (HET) knock-in mice carrying a *Mybpc3* point mutation (G>A transition) associated with HCM.

The LV mass was significantly lower in female WT and HET mice (23% in WT and 25% in HET), compared to corresponding male mice. Isometric force measurements revealed a significant lower maximal generated tension (F_{max}) in HET male (13.0 ± 1.1 kN/m^2), than in females (20.0 ± 2.2 kN/m^2). Exercise induced a higher fractional shortening in HET male mice, which is correlated with an increased F_{max} in exercised HET males. In contrast, LV weight was significantly increased in exercised HET females compared to sedentary females (7% in WT and 15% in HET). Ca^{2+}-sensitivity was increased in exercised male and females WT mice. Similarly, Ca^{2+}-sensitivity was enhanced in HET females, however not in exercised HET mice.

In conclusion, exercise training improved cardiac and myofilament performance particularly in HET male mice, indicating that physiological stimuli may elicit a sexually dimorphic cardiac response in heterozygous *Mybpc3*-targeted knock-in mice.

1750-Pos Board B480

Gender Differences in Passive Tension in Hypertrophic Cardiomyopathy Patients

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Background

Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disorder with a prevalence of 1:500. In ~65% of all HCM patients the causative mutation is identified. HCM patients that are sarcomere-mutation negative tend to have a less severe phenotype. Mutations in the *MYBPC3* and *MYH7* genes encoding cardiac myosin-binding protein C (cMyMP-C) and β-myosin heavy chain (MyHC) represent >80% of all genotyped HCM cases. HCM is characterized by asymmetric hypertrophy of the left ventricle and diastolic dysfunction. In the present study we investigated if passive stiffness of the sarcomeres may underlie diastolic dysfunction.

Methods

In-vitro passive tension measurements were done at sarcomere lengths of 1.8 to 2.2μm in cardiomyocytes from 10 sarcomere mutation-negative patients(SMN: 5 male, 5 female), 17 patients carrying a *MYBPC3* mutation(MYBPC3: 10 male, 7 female), and 10 patients carrying a *MYH7* mutation (MYH7: 5 male, 5 female). Tissue was obtained during myectomy surgery from the interventricular septum. Cardiomyocytes were mechanically isolated and Triton-permeabilized.

Results
Passive tension over the entire range of sarcomere lengths did not differ between sarcomere-mutation positive and mutation-negative male HCM patients. Passive tension in myocytes from sarcomere mutation-positive women was significantly higher compared to female mutation-negative HCM patients. Female MYH7 cardiomyocytes showed a higher sarcomere stiffness compared to male MYH7.
Conclusion
Our measurements suggest that high sarcomere passive stiffness may contribute to diastolic dysfunction in female HCM patients harboring a mutation in genes encoding thick filament proteins.

1751-Pos Board B481
Sex-Related Differences in Myosin Heavy Chain Isoforms of Human Failing and Non-Failing Atria
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Mammalian hearts express two myosin heavy chain (MHC) isoforms, which drive contractions with different kinetics and power-generating ability. The expression of the isoform that is associated with more rapid contraction kinetics and greater power output, MHC-α, is down-regulated, with a concurrent increase in the relative amount of the slower isoform, MHC-β, during the progression to experimentally-induced or disease-related heart failure. This change in protein expression has been well studied in right and left ventricles in heart failure models and in humans with failure. Relatively little quantitative data exists regarding MHC isoform expression shifts in human failing atria. We previously reported significant increases in the relative amount of MHC-β in the human failing left atrium. The results of that study suggested that there might be a sex-related difference in the level of MHC-β in the left atrium, but the number of female subjects was insufficient for statistical analysis. The objective of this study was to test whether there is, in fact, a sex-related difference in the level of MHC-β in the right and left atria of humans with cardiomyopathy. The results indicate that significant differences exist in atrial MHC isoform expression between men and women who are in failure. The results unexpectedly also revealed a two-fold greater amount of MHC-β in the non-failing left atrium of women, compared to men. The observed sex-related differences in MHC isoform expression could impact ventricular diastolic filling during normal daily activities, as well as during physiologically stressful events.

1752-Pos Board B482
Myocardial Infarction-Induced N-Terminal Fragment of Cmybp-C Impairs Myofilament Function in Human Left Ventricular Myofibrils
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Rationale: Myocardial infarction (MI) is associated with depressed cardiac contractile function and progression to heart failure. Cardiac myosin binding protein-C (cMyBP-C), a cardiac-specific myofilament protein, is proteolyzed post-MI in humans and results in an N-terminal fragment, C0C1f. The presence of C0C1f in cultured adult cardiomyocytes results in decreased Ca^{2+} transients and cell shortening, in addition to the induction of heart failure in a mouse model. However, the underlying mechanisms remain unclear.
Objective: To determine how C0C1f causes altered contractility in human cardiac myofilaments in vitro.
Methods and Results: We generated recombinant human C0C1f (hC0C1f) and incorporated it into skinned human left ventricular myocytes. Mechanical properties were then studied at sarcomere lengths of 2.0 and 2.3 μm. Our data demonstrate that the presence of hC0C1f in the sarcomere decreased maximal force myofilament Ca^{2+} sensitivity, increased cooperative activation at short lengths and enhanced length-dependent activation. Furthermore, hC0C1f led to increased cross-bridge cycling kinetics and tension cost at both short and long sarcomere lengths. We further established that the detrimental effects of hC0C1f occur through direct interaction with the thin filament proteins actin and α-tropomyosin (α-TM).
Conclusions: Our data demonstrate that the presence of hC0C1f in the sarcomere is sufficient to induce depressed myofilament function and Ca^{2+} sensitivity in otherwise healthy human donor myofilament preparations. Decreased cardiac function post-MI may result, in part, from the ability of hC0C1f to bind actin and α-TM, suggesting that cleaved C0C1f could act as a poison peptide and disrupt the interaction of native cMyBP-C with the thin filament.
Keywords: Cross-bridge cycling kinetics; length-dependent activation; cMyBP-C; C0C1f protein.

1753-Pos Board B483
Beta-Adrenergic Response in Human HCM Myocardium: Effects of Ranolazine
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Background: Rest or exercise obstruction is present in two thirds of patients with hypertrophic cardiomyopathy (HCM) and is a major determinant of symptoms and disability. Hypercontractile upper septum is the main pathophysiological determinant of obstruction, thus negative inotropic interventions such as dysopiramide or beta-blockers are the only available options to pharmacologically treat obstruction, commonly with partial efficacy. We have previously demonstrated that ranolazine ameliorates diastolic function in trabeculae from septal samples of obstructive HCM patients undergoing myectomy (Coppini et al, Circulation 2013).
Methods: Patch clamp studies and intracellular Ca^{2+} recordings were performed in isolated myocytes from myectomy samples of obstructive HCM patients; intact trabeculae were used for mechanical measurements. Myocardial specimens from non-failing non-hypertrophic patients or patients with secondary hypertrophy were used as controls.
Results: Dysopiramide (Dys) reduced twitch tension in a dose dependent manner and 5μM Dys accelerated contraction kinetics in HCM trabeculae. Isoproterenol 10^{-7} mol/L (Iso) determined a significant potentiation of twitch amplitude and an accelleration of contraction kinetics (both time to peak and relaxation). Changes induced by Iso in control trabeculae were similar. Interestingly, Iso caused APD prolongation in HCM cardiomyocytes instead of the shortening observed in control cells. This was likely related to the unbalance between depolarizing and repolarizing currents, including increased Late-Na^+ current (I_{NaL}). The I_{NaL} blocker Ranolazine 10 μM (Ran) applied on top of Iso (Iso+Ran) markedly reduced isometric twitch tension of HCM trabeculae, while Ran alone showed no negative inotropic effect. Contraction kinetics in Iso+Ran were still significantly faster compared to baseline.
Conclusions: Beta adrenergic stimulation may enhance septal contractility and determine obstruction in HCM. Ranolazine, by reducing septal tension at peak exercise but not at rest, may represent a safe therapeutic option for obstruction.

1754-Pos Board B484
Depressed Contractility at Low-Load Spontaneous Oscillatory Contractions in Human Hypertrophic Cardiomyopathy with MYBPC3 Mutations
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We examined the role of missense and nonsense myosin-binding protein mutations in human tissue of patients with hypertrophic cardiomyopathy in an essentially unloaded isotonic system by partial Ca^{2+}-activation, i.e. Ca-spontaneous oscillary contractions (SPOC). Despite considerable literature suggesting that hypercontractility is a feature of HCM-causing mutations, we observe: (1) prolonged durations of both the lengthening ($p<0.0001$) and shortening ($p<0.001$) phases of the SPOC cycle in MYBPC3 mutants; (2) depressed contractility where the rates of both lengthening ($P<0.01$) and shortening ($p<0.05$) were reduced; however (3) the amplitude of the SPOC cycles did not vary between mutated MYBPC3 and healthy donor samples under essentially unloaded isotonic conditions. We found no difference between MYBPC3 samples containing missense or nonsense mutations. Unexpectedly, principal component analysis demonstrated that the contractile properties of human derived cardiomyocytes under low-load conditions were distinctively different for mutations in MYBPC3 and troponin genes. We conclude that, at least under the isotonic